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Histology of the Skin of Some Toads and Frogs

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INTRODUCTION

Although the integument of the tailless amphibians is built on a fundamental plan (fig. 7), each species has its peculiarities. The epidermis varies in thickness; the basal layer may have a smooth basal surface, or it may be indented. On the surface, the epidermis may be smooth, or it may show projections and indentations. The stratum spongiosum of the corium may be thick or thin, and it may even be absent. The glands may show special arrangements. Finally, there are elevations and thickenings of many kinds, of which many are easily visible to the naked eye and contribute to the general appearance of the animal, while others are microscopic.

Many such peculiarities of European toads have been described by Leydig (1876a) in a paper unfortunately not illustrated. In 1931 Rabl gave a comprehensive account of everything known up to that time about special structures in amphibian skin. In 1937 Elias made a contribution to this subject.

In the present paper, special skin structures of a few species, most of them North American, are discussed. We consider the composition of the epidermis and the arrangement of glands, as well as the structure and arrangement of tubercles, warts, ridges, papillae, cones, and spines. A Latin name is given to each type, because it is frequently possible to describe a complicated structure in Latin with only a noun and an adjec-

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tive, whereas in English longer phrases or sentences are necessary to achieve the same end.

Because relatively few species have been investigated, the list of terms is, of necessity, incomplete.

Feet thickenings and thumb pads, though belonging to the skin elevations, are not included in the following discussion.

METHODS AND MATERIALS

The skin was observed in living and fixed specimens under the dissecting microscope, and valuable views were obtained by the use of an almost tangential direction of viewing. The arrangement of skin elevations can well be studied by this method.

The skin was preserved in sublimate or in formol, embedded in paraffin, and cut in serial sections 10 μ thick. These were stained with thyonin, toluidin blue, Delafield's hematoxylin and eosin, or with Hansen's iron hematoxylin and picrofuchsin.

For the demonstration of nerve endings Cohnheim's gold chloride and Bielchovsky's method were used.

For many of the specimens we are indebted to Mr. Charles M. Bogert of the American Museum of Natural History, to Dr. Arthur N. Bragg of the University of Oklahoma, to Dr. Albert P. Blair of the University of Tulsa, and to Dr. Bernard Greenberg of Roosevelt University, Chicago, Illinois, for both specimens and skin sections.

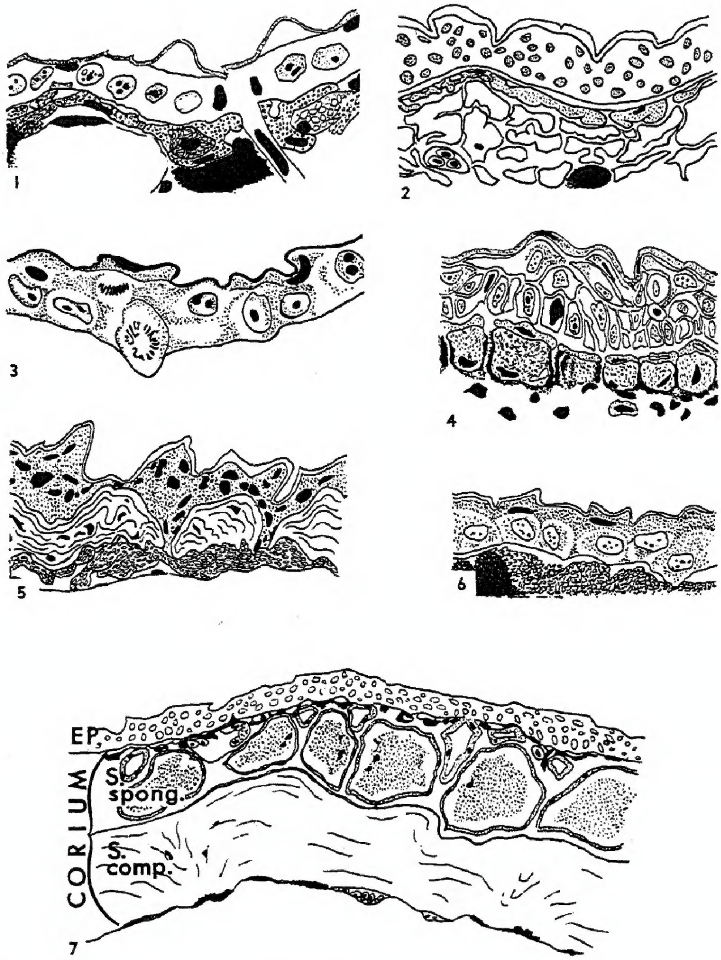
CLASSIFICATION OF SKIN THICKENINGS

Tuberculum Fluxum

The tuberculum fluxum is a small bump, visible to the naked eye. It was found in *Hyla gratiosa*. The loose skin of this animal contains numerous round bumps, which are flattened when the skin is stretched or when a glass plate is pressed on it. When the animal turns the head to the left, the tubercula fluxa (fig. 8) disappear in the neck region on the right.

Crista Glandularis

The skin of some members of the genus *Rana* is characterized by longitudinal ridges that separate the dorsal and lateral regions. Similar ridges are sometimes found in the dorsal region (*Rana pipiens*). They owe their existence to the presence of particularly large, granular glands, as has been shown by Gaupp (1904). Gaupp's illustration has been re-



FIGS. 1–7. Skin sections. 1. From dorsum of *Eleutherodactylus ricordi.* × 630. 2. *Scaphiopus holbrooki.* × 225. 3. From dorsum of *Eleutherodactylus ricordi,* section to show mitoses and method of cornification of epidermis. × 665. 4. From dorsal neck region of *Hyla gratiosa.* × 475. 5. From venter of *Hyla cinerea.* × 305. 6. From dorsum of *Hyla squirella.* × 475. 7. From venter of *Microhyla carolinensis.* × 150. *Abbreviations:* Ep., epidermis; S. comp., stratum compactum; S. spong., stratum spongiosum.

produced by Holmes (1938, fig. 59). In *Pipa pipa* a pair of dorsolateral and a pair of lateral rows of large compound warts correspond to the cristae glandulares of the frogs (fig. 14).

Crista Introversa Epithelialis

Cristae introversae epitheliales are long, epidermal ridges that project inward from the epidermis into the corium, as do the dental laminae of

mammalian embryos. A cross section of the crista introversa epithelialis medialis of *Bufo fowleri* is shown in figure 15.

Verrucae

Elevations of the skin have always been called warts (verrucae), regardless of their size (Leydig, 1876a). However, the macroscopic elevations in *Pipa* are not included among the verrucae for reasons stated below. The following types can be distinguished:

Verruca Glandularis: A wart that owes its existence to the presence in the skin of especially large, generally granular glands (figs. 16 and 24). Most of the warts shown in figure 27 are of this type. The parotoid "glands" are the largest verrucae glandulares.

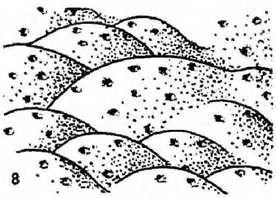
Verruca Syndesmica: A wart produced by a thickening of the corium (fig. 19). The stratum Malpighii and the replacement layer for the horn layer are not thickened.

Verruca Syndesmoepithelialis: A wart containing thickened corium and epidermis (fig. 17).

Verruca Epithelialis: A wart formed of epidermis only (fig. 25). In *Bombina variegata pachypus,* this is accomplished by the enlargement of the epidermal cells and of the intercellular spaces alone. In this species, there is no increase in the number of cell layers of the epidermis (Elias, 1937, fig. 7). Enlargement of the epidermal cells is always encountered in the verrucae epitheliales, but there can be, as is the case in *Bufo americanus,* an increase of the number of cell layers as well (fig. 22).

Verruca Epithelioglandularis: A wart containing a large granular gland, above which the epidermis is considerably thickened, as in figure 29. It appears, in the section demonstrated in this illustration, as if the corium did not participate in the formation of this wart; there is nevertheless present a small corium papilla, not hit by the knife in this section, but in a section 20 μ away from it (fig. 31). Its contribution to the bulk of the wart is insignificant. Its presence, however, is indicative of the possible existence of such warts as verrucae syndesmoepithelioglandulares.

Verruca Unicellularis: A wart which is actually a verruca epithelialis (figs. 18, 26) or verruca syndesmoepithelialis (fig. 20) of microscopic size. Through the proliferation of a single cell in the stratum Malpighii, a column of several lens-shaped cells is formed, which protrudes above the level of the epidermis. These warts were described by Maurer (1895) for *Triturus helveticus,* and by Dennert (1924) and later by Rotmann and Macdougald (1936) for *Triturus taeniatus.* Fig-



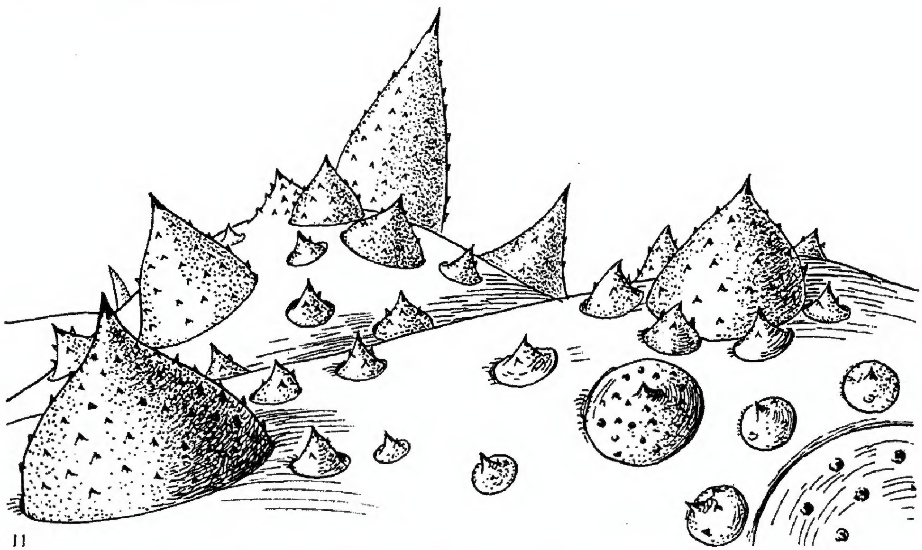


FIG. 8. Tubercula fluxa in parietal region, also openings of skin glands, of *Hyla gratiosa*. × 15.
FIG. 9. Dorsal skin of *Scaphiopus couchi*. × 25.
FIG. 10. Three coni circumfossati from dorsum of *Scaphiopus hurteri*. × 50.
FIG. 11. Skin of shoulder of *Pipa pipa* between angle of mouth and head of humerus, dorsal view, showing large and small coni circumfossati studded with verrucae unicellulares. Dorsal skin with broad cones in foreground; ventral skin with tall, thin conus circumfossatus in background. × 15.

ure 18 is redrawn after Dennert. Verrucae unicellulares exist also in toads (fig. 20, *Bufo americanus*, and in *Pipa pipa*).[1]

VERRUCA CORNEA: A microscopic wart consisting of stratum corneum

[1] According to the absence or presence of a small corium papilla, the verrucae unicellularis can be subdivided into verrucae epitheliounicellulares and verrucae syndesmounicellulares.

only. No special differentiation of the corium, or of the stratum Malpighii, or of the stratum granulosum or lucidum can be recognized under such a tiny wart. Figure 21 shows a wart of this type.

Denticulus Pluricellularis

This is an irregularly shaped, jagged projection of the epidermis made up wholly of epidermal epithelium (figs. 5, 33).

Denticulus Corneus

Minute projections have been described by Leydig (1876b). They are tiny jags of the stratum corneum, smaller than a single cell. Several of them may arise above one cell. Leydig found them in salamanders and on the thumb pads of toads. In Rabl's monograph (1931) Leydig's figures are reproduced.

Papilla

Gradual transitions exist between all types of warts, but there are other elevations that have a different appearance, and no transitions have as yet been observed between them and warts. Leydig (1876a) observed certain projections of the skin of *Bufo vulgaris,* which he called papillae because of their resemblance to the papillae of mammalian tongues. According to Leydig's description, the papillae he found seem to be papillae filiformes. They are slender and pointed and rather high in comparison to their width. They contain a slender corium papilla with a capillary loop (figs. 28, 35, 38, 39).

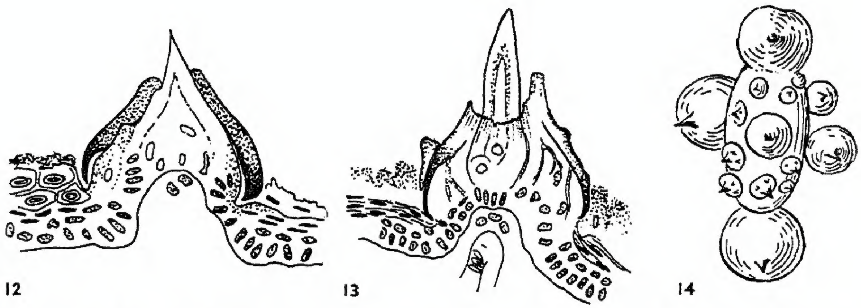
Conus Circumfossatus

The members of the genus *Scaphiopus* show projections basically of conical shape which have a black, apical spine of stratum corneum as in *S. couchii* (fig. 9). These cones are surrounded by a circular ditch (fossa) and, therefore, are given the name coni circumfossati. A corium papilla projects into the epidermal cone. This papilla contains a sensory organ (frequently a tactile corpuscle of the Meissner type, fig. 34) and sometimes also contains an innervated flask cell.

In *Pipa pipa* many of the coni circumfossati reach macroscopic dimensions (fig. 11). Because of their size, one might classify them among the verrucae, but the relation of some of them to larger verrucae glandulares puts them in the present class (see p. 8, paragraph 3).

Conus Apicalis

In many forms the apex of each wart and in *Pipa* the apex of each cone are crowned by a portion of the stratum corneum that is thickened



FIGS. 12, 13. Small coni circumfossati from dorsum of *Pipa pipa*. × 175.
FIG. 14. Oblong wart of lateral line of *Pipa pipa*, on which are five large coni circumfossati in regular pattern.

and frequently blackened but not surrounded by a fossa. The small, black stipples visible to the naked eye on the skin of common toads are such thickened and blackened horn portions. These coni apicales show all degrees of obtuseness and acuteness. If the angle at their apex is acute, they are called spinae (= coni apicales acuti) : they are otherwise called coni apicales rectangulares or obtusi.

SPINA (= CONUS APICALIS ACUTUS): A spina is a hard, sharp projection of the skin. Its core can be a corium papilla as in figure 16 (spina syndesmica), or it can consist wholly of epidermis as in figure 29 (spina epithelialis). The structures in question are actually small spines, whence the old name *Bufo spinosus* for *Bufo vulgaris*. Figure 23 shows spines in *Bufo regularis,* and figure 27 shows them in *Bufo americanus*. The sharpest spines observed occur on the large coni circumfossati of *Pipa pipa* (figs. 11–13).

CONUS APICALIS RECTANGULARIS AUT OBTUSUS: Not everywhere in *Bufo americanus* are the thickened coronations so acute as on the arm (figs. 16, 27, 29). There are transitions from this type of extreme acuteness to more obtuse forms such as are found in *Bufo fowleri* (fig. 28) or in *Bombina variegata* (= *Bombinator pachypus*) (Elias, 1936, fig. 7). In the latter case we can hardly speak of spines. In *Bufo fowleri* it is questionable as to whether these conical apices can still be regarded as real spines. In *Scaphiopus hurteri,* they are rounder, and in *S. holbrooki* those crowning large warts are completely flattened. Nevertheless, they are all homologous to the most acute of all spines. Transitions from the most acute to obtuse forms can be observed in each specimen of *Bufo americanus*. The comparison of figures 16, 19, 17, and 22 all taken from the same individual, shows this. It is difficult, if not impossible, to draw a line of demarcation between a real spine and an obtuse horny thickening of the apex of a wart or papilla.

THE SKIN OF INDIVIDUAL SPECIES

PIPA

Pipa pipa

Five preserved specimens were examined.

This animal possesses macrospopic and microscopic coni circumfossati (fig. 11) topped by sharp horny spines. The stratum corneum is yellow in color but red at the apices of the cones. The slopes of the cones are studded with horny verrucae unicellulares which are also reddish at their tops. The stratum corneum ends in a thick but sharp edge at the bottom of the surrounding fossa (figs. 12, 13).

Many, but not all, macroscopic cones are surrounded by a circle of microscopic cones (fig. 11, right). Along two lateral and two dorsolateral lines, larger warts (verrucae glandulares) are arranged in linear fashion. They are elliptic in shape (fig. 14), the long axis directed in a sagittal direction. The apex of each wart is occupied by a macroscopic conus circumfossatus. At each of the four ends of the major and minor axes of the base ellipse of each wart, there is one macroscopic cone. Thus, five macroscopic coni circumfossati are associated with each wart in regular fashion. Between them, numerous microscopic coni circumfossati are found in irregular arrangement.

At the ventral surface of the body, and particularly in the cervical region, the macroscopic coni circumfossati are twice as high as wide (fig. 11, background).

SCAPHIOPUS

The epidermis of *Scaphiopus* is free of any pigment. Freedom from pigment is a feature common to several primitive Salientia, such as *Discoglossus, Bombina,* and *Hyla.*

The epidermis is in most areas four to five cell layers thick, including the stratum corneum. Occasionally there are indentations of the epidermis where only one cell layer is found below the stratum corneum. The epidermal surface is wavy. The thickest areas show rounded elevations which are intermediate between denticuli pluricellulares and verrucae epitheliales (fig. 2).

The numerous macroscopic warts are verrucae syndesmicae. Their core is formed chiefly of stratum compactum. In these warts the glands are neither more numerous nor larger than elsewhere.

The only verrucae glandulares are the paratoid "glands."

The members of the genus *Scaphiopus* are provided with numerous microscopic coni circumfossati.

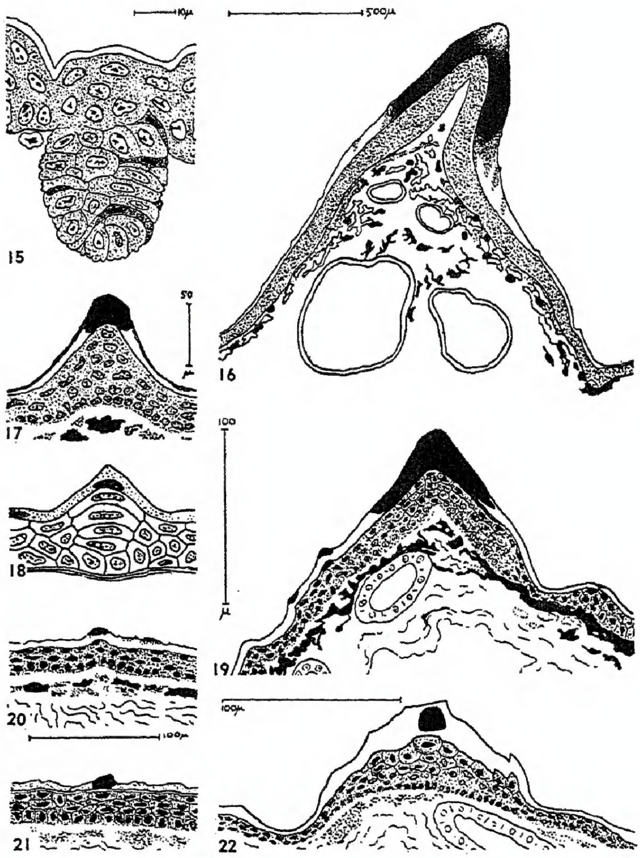


FIG. 15. Section of crista introversa dorsalis medialis of *Bufo fowleri*. × 475.

FIG. 16. Verruca glandularis of *Bufo americanus*. Same wart as shown in figure 27 15 mm. from right margin and 7 mm. from top margin of that illustration but narrower and apparently higher owing to the fact that the glandular contents were discharged when the animal was killed. × 30.

FIG. 17. Verruca syndesmoepithelialis from dorsum of *Bufo americanus*. × 140.

FIG. 18. Verruca epithelialis unicellularis of *Triturus taeniatus*. Redrawn after Dennert, 1924.

FIG. 19. Verruca syndesmica from dorsum of *Bufo americanus*. × 205.

FIG. 20. Verruca syndesmoepithelialis unicellularis from upper arm of *Bufo americanus*. × 150.

FIG. 21. Verruca cornea from dorsum of *Bufo americanus*. × 205.

FIG. 22. Verruca epithelialis from dorsum of *Bufo americanus*. × 205.

Scaphiopus hammondi

Four preserved specimens were examined.

The apex of each wart is occupied by a perfectly conical conus circumfossatus with a sharp, blackened, horny spine. This apical conus cir-

cumfossatus is surrounded by a circular area free of circumfossated cones and free of melanophores. Beyond the periphery of this circle, smaller coni circumfossati are scattered in irregular and rather even distribution. These cones are also present in the valleys between the warts. The sharp horny spines of the cones in the valleys are not blackened.

Scaphiopus couchi

Three preserved specimens were examined.

The coni circumfossati have a rather dull apical spine (fig. 9). These spines are blackened. The cones are rather evenly distributed, but the cone at the apex of each spine is larger than the others, and there is a gradual decrease in size from the apex of a wart to the interverrucal valleys.

Scaphiopus hurteri

Fifteen specimens (eight alive and seven preserved) were examined. In this species, the coni circumfossati vary greatly in distribution, shape, and size. We encounter among them short cylinders with rounded apex, hemispherical structures with rough surfaces (fig. 10), and even, flat disks. In many of them the stratum corneum ends with a sharp edge at the bottom of the surrounding fossa. In some specimens, the projections are high cylinders with rounded apices. The stratum corneum also varies in degree of blackening. The apical conus is always the largest.

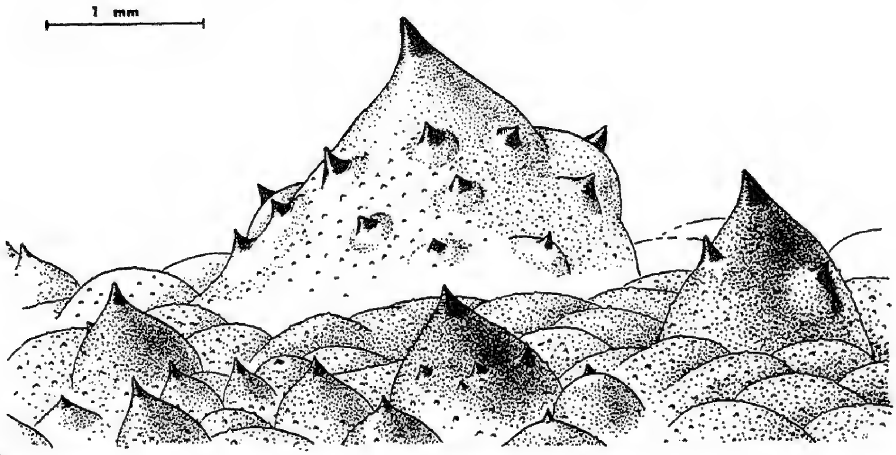
Scaphiopus bombifrons

Six preserved specimens were examined.

The apical structure in this species deserves the name of "conus" owing to homology rather than shape, for it is greatly broadened so that it becomes a macroscopically visible black dot. In addition, it is flattened, and its surface is wrinkled. It is surrounded by a circular area free from coni circumfossati, beyond which are small, rounded, circumfossated sensory organs, barely projecting beyond the epidermal surface. A small, nipple-shaped, horny spine crowns these small coni circumfossati.

Scaphiopus holbrooki

Twelve living and six preserved specimens were examined. The apical conus circumfossatus is almost completely degenerated. It is reduced to a large brown spot with hazy boundaries. The dark color is located within the stratum corneum, which is slightly thickened at this location. This spot on top of each wart is surrounded by a circular area free from coni circumfossati and free from melanophores. Beyond this circle the skin is studded with well-developed, pointed coni circumfossati with



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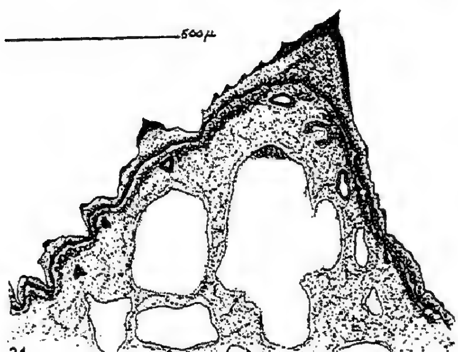

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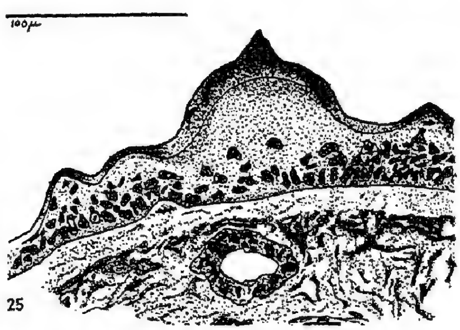

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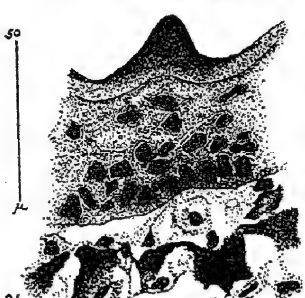

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FIG. 23. Lateral, thoracic skin of *Bufo regularis* male. × 18.5.

FIG. 24. Verruca glandularis of *Bufo regularis*, like verrucae in foreground of figure 23. × 205.

FIG. 25. Verruca epithelialis of *Bufo regularis* from slope of larger wart shown in figure 24. × 205.

FIG. 26. Verruca unicellularis from dorsum of *Bufo regularis*. × 400.

black tops. A conus circumfossatus, not sectioned through the apex, is shown in figure 34. This section, though not containing the apex of this cone, was chosen in order to show the tactile corpuscle in its corium papilla.

ELEUTHERODACTYLUS
Eleutherodactylus ricordi

Three living (subsequently preserved) specimens were examined. The epidermis of *Eleutherodactylus ricordi* is the thinnest yet found in adult Salientia. Its average thickness is 10 μ, but over large areas it measures only 7 μ in thickness. The stratum Malpighii almost resembles a simple cuboidal epithelium, the height of which is slightly less than the average width of one cell (fig. 1). In reality the epidermis has three cell layers: (1) the stratum Malpighii, consisting of rather low cuboidal cells; (2) the replacement layer (stratum granulosum), which is not a continuous layer, but consists of single cells or groups of cells pressed upward from the stratum germinativum and flattened; and (3) the stratum corneum, which measures 1 μ in thickness.

The mode of cornification of the upper layer and of its replacement can be studied in figure 3; two adjacent cells are seen in metaphase. This seems to indicate that repeated divisions occur here. One thing at least is certain; the upper dividing cell in the stratum granulosum can be situated here only as a consequence of a previous division, after which it has been pushed upward. This cannot have taken place long before, for the deposition of keratohyalin granules in its cytoplasm has not yet begun. The cell is still very much alive. The upper cell is in a phase which must be the second of two successive divisions, and as the large dividing cell in the stratum Malpighii is in the same phase of mitosis, it is probable that it is a sister cell of the upper one. Consequently it seems that repeated divisions take place here. The epidermis is much thicker than elsewhere at this point (18 μ, disregarding the downward bulge of the dividing basal cell). Perhaps the presence of areas with appreciably thickened epidermis is an indication that temporary centers exist for the production of stratum granulosum cells.

Keratinization in the distal cell layer takes place from above downward within these distal cells. This can be seen, in figure 3, in the cell just above the smaller dividing cell and in the distal cell at the extreme right. The part of the cytoplasm that faces outward is almost completely keratinized, while that part of the cytoplasm in contact with other cells retains the appearance of living cytoplasm with granules (probably of keratohyalin).

Smooth muscle cells connected with the epidermis can be seen in many areas (fig. 1). These cells run vertically through the corium and may be connected with cells of the stratum germinativum (as in fig. 1), or with cells of the stratum corneum. No pigment is found in the epidermis.

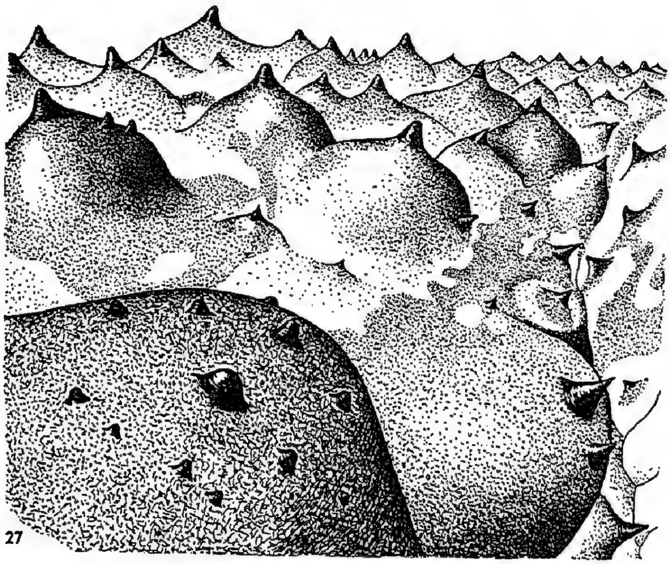

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FIG. 27. Skin at bend of right elbow of *Bufo americanus.* × 10.
FIG. 28. Optical section of shed stratum corneum of a medium-sized wart from dorsum of *Bufo fowleri.* × 42.

BUFO

Bufo americanus

Thirteen living (subsequently preserved) specimens were examined. Dickerson (1931, fig. 61, pl. 22, facing p. 83) shows "the resemblance of the toad's skin to garden earth, both in color and texture." This resemblance in texture to garden earth derives from the warts of all sizes that are present. The roughness of the skin is increased by the presence of small, black spines, visible to the naked eye. The largest spines are found on the apices of warts and particularly on the arms (fig. 27). The presence of so many warts of all sizes is thought to produce protective resemblance to soil, and the presence of the spines may discourage predatory enemies. In addition, the warts and spines may have a more important function; some, if not all, seem to be tactile organs, as is demonstrated below.

Figure 27 shows the skin at the volar surface of the elbow. The long groove running vertically along the right margin is the bend of the elbow. The figure shows many warts—large, medium-sized, and small. All warts are drawn to scale. The medium-sized warts in the background are in reality much smaller than those in the foreground.

Many large- and medium-sized warts in this figure bear a spine at the apex. Near the right lower corner of the figure is a wart with two spines. The large wart in the left foreground bears at its apex not a spine but what appears to be a wart of the type shown in figure 17. On the same large wart 11 smaller obtuse warts can be seen.

At the left margin of the picture appears a wart which is provided with two small, spined warts, in addition to an apical spine. Additional medium-sized and small warts can be seen in the "valleys" between the large warts. Microscopic warts, though present everywhere, cannot be seen with this moderate enlargement.

Figure 37 represents two very irregularly shaped verrucae syndesmoepitheliales. The one at the left shows several unicellular warts growing on its slope.

Leydig (1876a) described types of warts in *Bufo bufo* quite similar to those of *Bufo americanus*.

PAROTOIDS: The parotoid "glands" are huge, flat verrucae glandulares, bearing no spines and with a relatively smooth surface. Only very few small, flat verrucae epitheliales (sometimes with corpus papillare, fig. 36) are present; papillae filiformes are present only where black spots invade the parotoids.

LARGE WARTS: All the large warts are verrucae glandulares.

MEDIUM-SIZED WARTS: The warts of medium size are verrucae glandulares.

SMALL WARTS: The small warts can be verrucae glandulares, verrucae epithelioglandulares (fig. 29), verrucae syndesmicae (fig. 19), verrucae syndesmoepitheliales (fig. 17), or verrucae epitheliales (fig. 22). Some of the verrucae epitheliales contain a corpus papillare (fig. 36).

MICROSCOPIC WARTS: In this order of magnitude we find verrucae epitheliales, verrucae unicellulares (left slope of left wart of fig. 37), verrucae syndesmo-unicellulares (fig. 20), and verrucae corneae (fig. 21). The verrucae unicellulares of *Bufo americanus* rarely have the beautiful regularity in their inner construction as do those described by Dennert for *Triturus taeniatus*.

SPINES: Most of the large- and medium-sized warts are crowned with a spine, as can be seen in figure 27. There are two kinds of spines: (1)

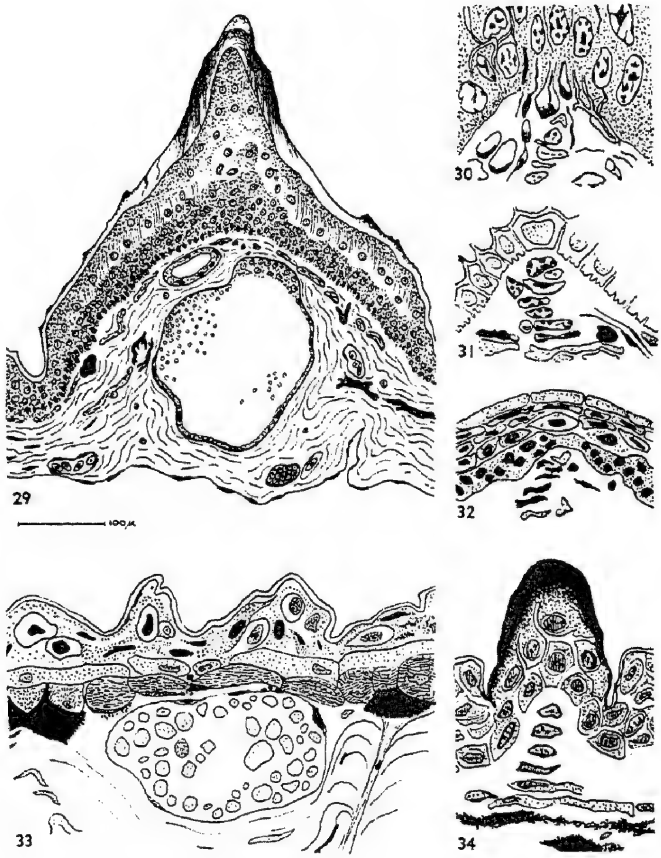


FIG. 29. Verruca epithelioglandularis from dorsum of *Bufo americanus*. × 98.

FIG. 30. Tactile corpuscle in a verruca syndesmica of *Bufo americanus*. × 500.

FIG. 31. Tactile organ of wart of *Bufo americanus* shown in figure 29, but located 20 µ from the section there shown. Hansen's iron hematoxylin, Van Gieson's picrofuchsin. × 500.

FIG. 32. Tactile corpuscle below a very low skin elevation of venter of *Bufo americanus*. × 233.5.

FIG. 33. Dorsal skin of *Hyla cinerea*. × 475.

FIG. 34. Papilla circumfossata with tactile corpuscle from dorsum of *Scaphiopus holbrooki*. × 430.

spina syndesmica, which contains a slender corium papilla (fig. 16), and (2) spina epithelialis, which is made up of solid epidermis (fig. 29).

At the apex of a spine the stratum corneum reaches a thickness of 60 µ or more. The side walls of its horny covering are at least 45 µ in thickness. The stratum corneum is blackened here.

Except for the extreme apex, the horn layer is black (figs. 16, 29).

Leydig (1876a) found that this is also the case in *Bufo bufo* and *Bufo viridis*.

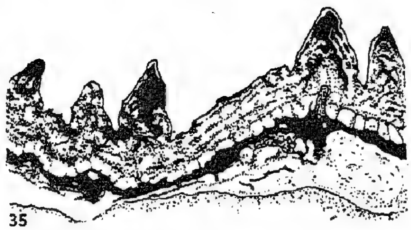
In addition, Leydig observed that some warts of *Bufo bufo* in a lateral position bear at their apices horny combs instead of spines.

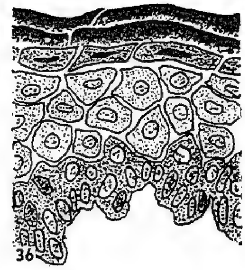
PAPILLAE FILIFORMES: The black spots of the American toad are provided with microscopic papillae filiformes. The epidermal cells within them are intensely pigmented with melanin granules. These are chiefly found distal to the nucleus, as is usually the case in pigmented epithelial cells of amphibians. The stratum corneum at the apex is not thickened. The papillae project 30 to 40 μ above the level of the epidermis and are about 50 μ wide at their base. They are heavily pigmented and impart a velvety appearance to the black spots. In some specimens we find verrucae syndesmo-unicellulares instead of the papillae filiformes.

CHRISTA INTROVERSA: An irregular white stripe runs along the median line of the back. Through the middle of the stripe runs a very delicate grayish line. The presence of this line is due to the fact that the chromatophores of this region, predominantly guanophores, are pushed downward by an epithelial ridge projecting from the epidermis into the corium. We call such a ridge crista introversa epithelialis. Figure 15 shows a cross section of the crista introversa of *Bufo fowleri*. This is not appreciably different from the crista introversa in *Bufo americanus*. It is therefore not necessary to give a separate illustration for the latter species. At this moment we cannot offer any explanation as to its significance.

WARTS AND SPINES AS SENSORY ORGANS: In the apex of the corium papilla of verrucae syndesmicae there is frequently found a column of flat cells that resembles a tactile corpuscle (fig. 31). This corpuscle is approached by what seem to be non-myelinated nerve fibers.

In a verruca epithelioglandularis we found a rather complicated arrangement of cells (fig. 30): myelinated nerve fibers approach the epidermis and penetrate it. Other myelinated nerve fibers lose the myelin sheath near the epidermis; they branch and send their branches into the epidermis. In addition, the corium papilla contains a column of flattened cells suggesting a tactile corpuscle. Finally, there are numerous round cells in the corium papilla, each of which seems to contain a large vacuole and a flattened nucleus pressed towards the basal wall of the cell. These cells may also be related to the sense of touch. (Unfortunately, these observations were made only on routine material stained with thionin alone or with Hansen's iron hematoxylin and Van Gieson's stain, and the observations are only preliminary. It seems, however, very plausible that these projections with their sharp point can be receptors for touch.)





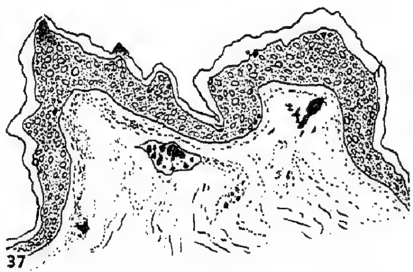


FIG. 35. Papillae filiformes in a dark spot from dorsum of *Bufo fowleri.* × 140.

FIG. 36. Section of skin on parotoid "gland" of *Bufo americanus*, showing a double-layered stratum corneum and the corpus papillare. × 333.

FIG. 37. Two very irregularly shaped warts from the lateral neck region of *Bufo americanus.* × 205.

FIG. 38. Papillae filiformes on slope of a large wart from dorsum of *Bufo fowleri.* × 53.5.

FIG. 39. Papillae filiformes and chromatophores of *Bufo fowleri.* At left: section through median white stripe; towards center: green border of a black spot; at right: interior of a black spot. In stratum vasculare of corium: white, guanophores; stippled, xanthophores; black, melanophores. × 53.5.

In addition to the cell columns within warts, the same kind of corpuscles can be found at places where there are no warts, but only slight, almost imperceptible elevations of the skin (fig. 32). These latter corpuscles are probably identical with what Maurer (1895) called "flache Tastflecke" which he described in an unidentified species of *Bufo* (probably *B. bufo*).

Merkel (1880) demonstrated tactile corpuscles of the same kind below verrucae epitheliales of *Rana esculenta* and in the corium papillae of verrucae syndesmicae; still others were found in flat arrangement below

the epidermis, where no thickening occurs. One of these types has been redrawn by Holmes (1938, fig. 104).

THE EPIDERMIS AND THE STRATUM CORNEUM: The basal boundary of the epidermis is not smooth, but the cells show indentations and projections (fig. 31). The same appearance of this layer has been described for *Rana esculenta* by Schulze (1867), for *Bufo viridis* (*variabilis*) and *Bombina bombina* (*Bombinator igneus*) by Leydig (1876b), and for *Necturus maculatus* by Dawson (1920).

The stratum Malpighii of *Bufo americanus* shows distinct intercellular spaces crossed by intercellular bridges. Tonofibrils can be demonstrated with iron hematoxylin. The stratum corneum is connected with the replacement layer by intercellular bridges which contain, of course, parts of tonofibrils.

According to Schulze (1867) and Schuberg (1893), only one cell layer of the stratum corneum is shed at each molting, while the second layer of keratinized cells remains connected with the epidermis until the next layer is cornified. Schuberg describes this for *Bombina bombina, Bufo bufo,* and *Rana esculenta.* Only occasionally, he says, are two layers shed.

In many cases, the stratum corneum of *Bufo americanus* includes two layers of cells before and during shedding. This is evident in figure 29 at the top of the spine. After molting, the next two cell layers cornify. In one case, however, we found the condition described by Schuberg. In this specimen the stratum corneum consisted of two distinctly separated layers (fig. 36). The third layer of cells was a stratum lucidum. Only the uppermost layer was shed, while the second still adhered to the stratum lucidum (fig. 32). This was the case in the specimen that possessed verrucae syndesmounicellulares instead of papillae filiformes.

In the majority of cases, the two upper cell layers are entirely cornified and very closely connected, and both are shed together.

The next molting probably does not occur before the second layer is cornified. In most cases, the two layers become so closely united that they appear homogeneous.

The stratum corneum plays an important role in the formation of the coni apicales. Towards the apex of every type of wart, except the parotoid, the horn layer becomes gradually thicker. As we have seen, it can attain a thickness of 60 μ in spines. At the apex of a certain verruca syndesmica (fig. 19) it measures 25 μ in thickness. It is then thicker than the entire living part of the epidermis which measures 20 μ in thickness at this place. In the verrucae epitheliales and syndesmoepitheliales it is less thick (up to 15 μ), while the living part of the epidermis is 50 μ thick or thicker (fig. 17). In the verruca epithelioglandu-

laris of figure 29, the stratum corneum is only 20 μ thick while the living part of the epidermis measures 100 μ in thickness. At the apices of the verrucae unicellulares the stratum corneum may become 10 μ thick, and in the verruca cornea it may reach 15 μ in thickness.

In some individuals, such as the one from which figure 29 was drawn, the replacement layer of the horn is the thickest of all cell layers. The cells of this layer are particularly large towards the apex of the wart and spine. Thus, one understands the thickening of the stratum corneum. But in most other cases, the thickening of the horn layer is not preceded by a thickening of the stratum granulosum or of the layer which takes its place. On the contrary, in most cases the replacement layer is made up of rather flat cells (see fig. 19), and the stratum corneum is often thicker than the entire living part of the epidermis under it. It is rather surprising to find a thickening of the horny layer in the verrucae corneae, where neither the epidermis nor the corium shows any thickening or other modification (fig. 21). At the apex of the verruca syndesmica of figure 19, the replacement layer is but 6 μ thick, while the horn layer above it measures 25 μ in thickness. This indicates that cornification of a cell layer is accompanied by swelling to more than double its thickness. It is remarkable that the thickening occurs only at these special locations, while the average thickness of the stratum corneum is 5 μ.

Equally surprising is the blackening of the apical portions of the horn layer. The black color does not derive from pigment in the epidermal cells,[1] for the epidermal cells at the apices of the warts and spines are not pigmented (figs. 16, 17, 19, 21, 22, 29, 34). Thus the black color of the stratum corneum of coni apicales does not have a visible precursor in the living epidermis. However, the blackening of the horn is preceded by a basophilic reaction of the keratin. The dark areas in the stratum corneum in figures 22 and 37 were colorless before the section was stained with thionin blue. The darkness of the horny layer seen in these drawings represents the blue stain. In sections fixed with sublimate, whether or not they are deparaffinized and covered with Canada balsam, the black color fades within a few months. Also, in this respect this black pigment differs from the granular melanin found in melanophores and in epithelial cells. Also, it is not of a granular character but blends gradually with the colorless surrounding and appears optically homogeneous.

Individual Differences: We have found specimens in which the

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[1] Except at the tops of papillae filiformes and of those verrucae syndesmounicellulares which can substitute for the papillae filiformes, but here the horn layer is not thickened.

majority of the smaller and medium-sized warts were formed by the corium only, and in which the stratum Malpighii at the apex of the warts was even thinner than elsewhere.

On the other hand, specimens were seen in which the epidermis played the predominant role in the formation of the smaller and medium-sized warts. In these specimens we did not find a single verruca syndesmica.

Again, some specimens show a clear arrangement of the epidermal cells in layers, while others show them irregularly arranged. In some animals, the replacement cells for the horn layer are almost columnar, i.e., much thicker than those of the underlying layers (40–45 μ high in the warts), while in others they are flat (up to 6 μ thick).

Most specimens possess papillae filiformes throughout the dark spots, while a few have verrucae syndesmounicellulares in their stead.

Bufo fowleri

Fifteen living (subsequently preserved) specimens were examined.

The skin structures of this toad resemble very much those of *Bufo americanus,* but there are differences. The coni apicales are never acute and cannot be called spines, but are rather coni apicales rectangulares or obtusi. The coni apicales, though the angles at their tops do not exceed a right angle, are nevertheless sharp (fig. 28).

The papillae filiformes are much more numerous and are higher than in *Bufo americanus.* In the black areas the papillae filiformes project 70 μ above the level of the epidermis (figs. 28, 35, 38, 39); this indicates that they are more than twice as high as those of *Bufo americanus.* A consequence is that the black spots of *Bufo fowleri* appear much darker and more velvet-like. *Bufo fowleri* possesses papillae filiformes also in light areas and even in the light median stripe on the back (left part of fig. 39). But at this location they are lower and not pigmented.

Like *Bufo americanus, Bufo fowleri* is provided with a median crista introversa, a cross section of which is shown in figure 15.

Bufo regularis

Two male preserved specimens were examined.

Among all the animals surveyed in this study, male specimens of *Bufo regularis,* an African toad, offer the most spectacular views of skin elevations (fig. 23). The skin is covered with low, rounded elevations (probably tubercula fluxa). Among them, one finds large, steep verrucae glandulares, of which figure 23 shows eight. They measure 0.3–1.3 mm. at their bases and are 1–1.5 mm. high. One is shown in section on figure

24. Verrucae epitheliales (fig. 25) are often found on their slopes. Finally, there are very large verrucae glandulares, measuring at their bases from 1.5 to 3 mm. in diameter and 1.8 to 2 mm. in height. The slopes are studded with verrucae epitheliales. One of these large warts is seen in the background of figure 23. Except for the apical regions of the verrucae, the skin of this animal is covered with numerous verrucae unicellulares (fig. 26). All these warts are irregularly and rather uniformly distributed over the dorsal and lateral surfaces of the toad. The distances between the very large warts range from 2 to 6.5 mm., those between the smaller warts from 0.15 to 2.3 mm. The average distance between two adjacent verrucae unicellulares is about 100 μ.

In the regions free of verrucae, the stratum corneum measures from 2 to 5 μ in thickness, the stratum Malphigii 8 to 20 μ. The epidermis contains two to three cell layers. The stratum spongiosum varies in thickness from 15 to 40 μ, while the stratum compactum ranges from 95 to 150 μ in thickness. The epidermis attains a thickness of 25 μ, the corium a thickness of 190 μ. Thus, in these regions, the skin may reach a thickness of 215 μ. Females have much lower warts. There is a clear sexual dimorphism expressed in skin surface structure (personal communication from Greenberg).

HYLA

Hyla cinerea

Eight living (subsequently preserved) specimens were examined. The dorsal epidermis of *Hyla cinerea* has a jagged surface. Its basal contour is practically straight (fig. 33). The epidermis consists of three cell layers throughout. The outermost of these three layers is the stratum corneum which is of uniform thickness (1.8 μ). The two lower layers represent a mixture of germinativum and granulosum cells. The arrangement of the cells is very irregular, and their shapes are varied. The granulosum cells are always in contact with the stratum corneum, but reach at some places down to the basal boundary of the epidermis (second depression from the left in fig. 33).

Denticuli pluricellulares are formed through this irregular arrangement and the variations of shape and position of the epidermal cells. In the depressions between them, the epidermis measures only 7 μ in thickness, including the stratum corneum. In the center of the denticuli pluricellulares the epidermis measures 20 μ in thickness. The difference in thickness is a matter of position and shape of the single cells, for there are everywhere exactly two layers of cells below the stratum corneum. In the depressions, the cells are flat and horizontally situated; in the

denticuli, they approach a columnar shape, with their long axes directed obliquely or vertically.

The stratum spongiosum is very thin (12–16 μ). It is entirely occupied by the chromatophores. The stratum compactum is pushed downward only in the regions of the glands. The stratum compactum is approximately 45 μ thick. The entire skin measures about 75 μ in thickness.

Both types of glands are scarce and are located at the same level.

On the ventral surface (fig. 5), the guanophores (or leucophores), which in many other forms are found in the stratum spongiosum and in the subcutis, occupy exclusively a subcutaneous position. The stratum spongiosum is entirely absent, and the stratum compactum touches the epidermis immediately.

In the ventral epidermis, the arrangement of cells is much more irregular than at the dorsal side. High denticuli, about 35 μ thick, alternate with extremely thin areas, which consist almost exclusively of the thin stratum corneum. At these places the epidermis measures only 2 to 2.5 μ in thickness (left, in fig. 5). At many places (right half of fig. 5) the epidermis reaches down to the subcutis.

In 1894 Maurer showed that smooth muscle cells arise from epidermal epithelial cells in *Rana esculenta, Rana temporaria, Triturus cristatus,* and *Rana agilis*. These smooth muscle fibers form the "perforating bundles" which penetrate through the stratum compactum. Schmidt (1918) described the connection of these muscle fibers with the epidermal cells by means of a fibrillar structure, the "cell tendon," in *Hyla arborea*. He doubts the epidermal origin of the perforating bundles, but does so without presenting any evidence of his dissenting opinion, merely stating that Maurer's evidence is insufficient.

The continuity of the epidermis in the ventral skin of *Hyla cinerea* through the corium down to the subcutis supports Maurer's (1894) and Weiss' view (1916) of the epidermal origin of the perforating bundles. Whether these perforating bundles of *Hyla cinerea* contain only epithelial cells, or myoepithelial cells and plain muscle cells, still remains to be determined.

The epidermis of *Hyla cinerea* is entirely free from pigment.

Hyla gratiosa

Twenty-five living (subsequently preserved) specimens were examined. The skin of *Hyla gratiosa* (fig. 4) is slightly thicker than that of *Hyla cinerea*. The total thickness of the skin is approximately 60 μ. The epidermis consists of four layers of cells, including the stratum corneum. Its surface is slightly jagged. The cells of the basal layer are columnar

and stand always in a vertical position. The denticuli pluricellulares are less numerous and less pronounced than in *H. cinerea.* At the thinnest places the epidermis measures 12 μ and at the thickest places 16 μ. This means the greatest height of a denticulus above the surrounding surface is only 4 μ (as compared with 20 μ on the ventral skin of *Hyla cinerea.*) The stratum spongiosum is as thin as in *Hyla cinerea* (approximately 15 μ). It is at most 2 μ thicker than the layer of chromatophores, but here also the stratum compactum is pushed downward where glands are located.

The granular glands measure up to 50 μ in width, and the mucous glands are up to 25 μ wide. The glands are separated by considerable non-glandular areas. Both types are located at the same level, i.e., immediately below the melanophores.

The stratum compactum measures about 30 μ in thickness.

Figure 8 shows a view of a part of the skin of *Hyla gratiosa,* 15 times enlarged; the skin is "tuberculated," showing numerous "bumps" which are tubercula fluxa; the small black dots scattered over the figure are glandular openings; the epidermis is entirely free from pigment.

Hyla squirella

A single specimen of *Hyla squirella* was available. The total thickness of the skin is about 60 μ. The epidermis is about 11 μ thick. It consists of three layers of cells (fig. 6). The stratum Malpighii is a single layer of "cuboidal" cells. The second layer is the stratum granulosum. Above this follows the extremely thin stratum corneum, only 1 μ thick. The surface has small irregularities. The area shown in figure 6 is one of the most jagged. Elsewhere, the surface is smooth, but shows several narrow identations, about 2 μ deep. Denticuli pluricellulares, as found in the other two hylas, are absent.

The lower surface of the epidermis is slightly irregular; a distinct basal membrane ("Grenzlamelle"), about 1 μ thick, is present.

The stratum spongiosum is approximately 12 μ thick and is almost completely filled by the chromatophores. The stratum compactum measures about 38 μ in thickness.

As in all other hylas, including *Hyla arborea,* the epidermis is not pigmented and does not contain epidermal melanophores.

RANA

In this genus the skin is usually smooth, but there are exceptions. Merkel (1880), Maurer (1895), and Krause (1923) describe small verrucae epitheliales in *Rana esculenta.* Rabl (1931) describes little

warts in *Rana catesbeiana* which are intermediate between verrucae epitheliales and verrucae unicellulares. All these warts are microscopic.

The skin of *Rana clamitans* is "tuberculated" with verrucae syndesmicae. Twenty specimens were examined. Other frogs have small but macroscopically visible warts, but nothing is known about their internal structure. The majority of the members of this genus possess dorsolateral ridges. *Rana pipiens* has, in addition, two somewhat less prominent ridges along the back between the dorsolateral ridges. These ridges are cristae glandulares, formed by large granular glands, as has been shown by Gaupp (1904).

MICROHYLA

A single specimen of *Microhyla carolinensis* was available for study (fig. 7). The epidermal surface is smooth. The stratum corneum is like that of *Rana*. The epidermis consists essentially of three layers of cells. The stratum Malpighii is one layer thick. The cells of the second layer are found to be in any condition between that of fresh cells such as those of the germinative layer (stratum Malpighii) and typical stratum granulosum cells. The third layer shows all stages of cornification, i.e., it contains flattened nuclei at some places, while at other places no nuclei can be seen. The epidermis is 18 to 25 μ thick.

The entire stratum spongiosum (fig. 7) is literally filled with two kinds of glands, regularly arranged in two layers. In the upper layer of the stratum spongiosum, the stratum vasculare, we find small mucous glands, lined by a low cuboidal epithelium. They measure 25 to 30 μ in horizontal diameter. They may be slightly oblong in the vertical direction, reaching down into the interstitial spaces between the granular glands as much as 60 μ. The mucous glands in the specimen at hand are slightly compressed by the large granular glands. The lower two-thirds of the stratum spongiosum ("stratum glandulare," fig. 7) is filled with large granular glands. These measure about 70 μ in diameter. The granular glands form a continous layer. They are separated from one another only by narrow spaces and press on one another with their side walls like the cells of an epithelium. Distally, they are practically in contact with the chromatophore layer and with the mucous glands. Basally, they are pressed towards the upper surface of the stratum compactum. With very little connective tissue present in the stratum spongiosum, the shape of the glands is determined by the pressure which they mutually exert on one another, by the outline of the stratum compactum, and by their limits above. In the specimen under investigation, the upper surface of the granular glands was dome-like as they were filled with

secretion; the mucous glands were empty. It can be assumed that when the mucous glands are filled they make their impression upon the granular glands. It is probable that figure 7 shows a rather abnormal condition, because the animal to be photographed died after 15 minutes of exposure to the air and strong electrical illumination. It can be assumed that under these conditions of dessication the mucus was discharged from the glands. Normally, in cool and moist environment the glands might be more or less filled and therefore more turgid than those shown in figure 7. We know of no other amphibians with a similarly dense layer of glands in the skin.

As far as observed, the granular glands of this frog resemble in every cytological respect the granular glands of the salamander, *Necturus maculosus*, described by Dawson (1920).

The stratum compactum is, on the average, 75 μ thick. The total thickness of the skin is about 170 μ. This is a remarkable thickness for such a small animal. Our specimen measured only 22 mm. in length. How remarkable this is becomes evident if one considers that the skin of the common toad, *Bufo americanus*, is only 100 to 140 μ thick. The common toad measures 9 cm. on the average, i.e., it is about four times longer, 64 times heavier, and it is known for the toughness of its skin. The other extreme is *Hyla gratiosa*, in which a specimen of about 6 cm. in length has a skin only 60 μ thick. This animal is 2.7 times longer and about 20 times heavier than *Microhyla carolinensis*, and its skin is one-third as thick.

The subcutis of *Microhyla carolinensis* contains numerous melanophores. The epidermis is not pigmented. These two factors are more noteworthy than the thickness of the skin, because this brevicipitid, generally considered as one of the most specialized of the Salientia, shows two distinctly primitive characters: absence of epidermal pigment and the persistence of subcutaneous melanophores, hitherto known only in the Discoglossidae (Elias, 1936, 1939, 1942), the Hylidae (Elias, 1937, and above), and the Pelobatidae (above).

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